reported 2 of 39 *C. texanus* to be infected with *Atractis penneri.* Specian and Ubelaker (1974) found *C. texanus* to be a host for *Parathelandros texanus* Specian and Ubelaker, 1974, but gave no additional details. *Thubunaea iguanae* is the third nematode species to be recovered from *C. texanus.*

One species of nematode has been previously reported from *H. maculata.* Gambino and Heyneman (1960) reported 23 of 48 *H. maculata* to be infected with *A. penneri. Physaloptera* sp. is the second nematode species to be recovered from *H. maculata.*

One species of cestode has been previously reported from *Cophosaurus texanus texanus.* McAllister (1988) reported 1 of 21 *C. texanus texanus* to be infected with *Mesocestoides* sp. Vaillant, 1863. *Oochoristica* sp. is the second cestode species to be recovered from *C. texanus.*

Although juvenile acanthocephalans have been reported occasionally from lizards collected in Arizona, this is apparently the first report of acanthocephalans from *C. texanus.* Benes (1985) reported 4 larval acanthocephalans from the coelom of 1 *Cnemidophorus tigris septentrionalis.* Goldberg and Bursey (1990a) found a juvenile acanthocephalan among the stomach contents of 1 *Cnemidophorus uniparens.* On another occasion, Goldberg and Bursey (1990b) recovered 3 unattached juveniles in the small intestines of 3 *Sceloporus jarrovii jarrovii.*

We thank Rana Tawil for assistance in removal of parasites.

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J. Helminthol. Soc. Wash.
59(2), 1992, pp. 231–233

Research Note

Grenacher's Borax Carmine for Staining Nematodes Inside Insects

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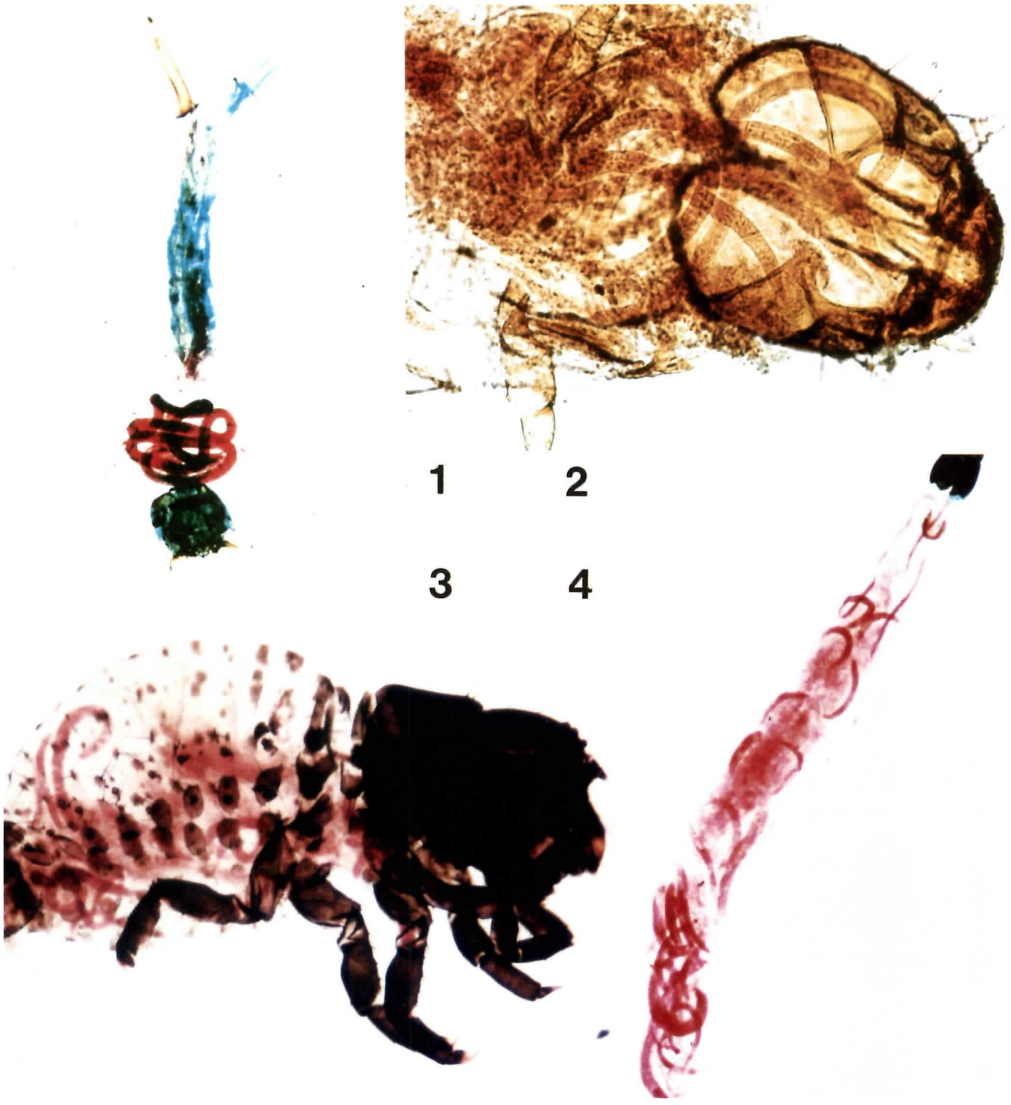
ABSTRACT: Grenacher's borax carmine stain was used to stain nematode parasites inside insects. This simple and useful procedure is described in detail. Examples of mermithid, *Romanomermis culicivorax,* and steinernematid nematodes, *Steinernema carpocapsae* and *S. feltiae,* stained well. The insects used were larval stages of both dipteran and coleopteran insect pests.

KEY WORDS: Nematoda, insect parasites, staining method, Grenacher's borax carmine, *Romanomermis culicivorax, Steinernema carpocapsae, Steinernema feltiae.*

During investigations on biological control of pest insects using nematodes, a need developed to show presence, size, and locations of nematodes inside bodies of insects. Usually insect lar-



Figures 1–4. Light micrographs of insect larvae parasitized by nematodes and stained with borax carmine. 1. The mosquito larva, *Culex pipiens quinquefasciatus,* parasitized by the mermithid nematode, *Romanomermis culicivorax.* 2. The western corn rootworm larva, *Diabrotica virgifera virgifera,* parasitized by the steinernematid nematode, *Steinernema carpocapsae.* 3. The Colorado potato beetle larva, *Leptinotarsa decemlineata,* parasitized by the steinernematid nematode, *Steinernema carpocapsae.* 4. The mushroom fly larva, *Lycoriella mali* parasitized by the steinernematid nematode, *Steinernema feltiae.*

vae are opaque because of high fat content. Therefore a technique was developed to clear the parasitized insect and to stain and destain nematode parasites.

The basic stain used in this procedure was the alcoholic borax carmine of Grenacher (1879) prepared using the recipe of Davenport (1960): 1.0 g carmine, 2.0 g borax, and 50.0 ml water.

Boil in a covered vessel for 30 min, or until the carmine dissolves; then add 50 ml of 70% ethyl alcohol (ETOH). Allow the solution to stand 1–2 days and filter through filter paper. The filtrate is then ready for use.

1. *Staining.* Begin procedure with parasitized insect larvae that have been fixed in 70% ETOH for at least 24 hr. Small holes made with a fine needle in bodies of corn rootworm and potato beetle let the stain go in efficiently. Transfer spec-

imens directly from 70% ETOH into Grenacher's borax carmine and leave overnight for at least 12 hr. Remove specimens from stain and rinse excess stain with several changes of 70% ETOH. A modified beem capsule (Day, 1974) was used during staining procedures and dehydration to avoid breaking the insect specimens during transfer. Both open ends of the tube were covered with nylon screen and then lids with holes cut in the center were placed on them.

2. *Destaining.* Destain in acid alcohol (70% ETOH + 2% HCl) until the nematode can be seen inside the insect. Destaining may be hastened by raising the HCl to 5%. Concentrations of HCl above 5% can destroy specimens. In the case of the mushroom fly, destaining did not take more than a few seconds. The potato beetle and corn rootworm took about 5–20 min depending upon the amount of fat in the insect. Transfer to plain 70% ETOH for overnight or weekend storage if destaining takes longer than a day. Stop destaining when the specimen becomes light pink and the darker nematode is visible inside. Destaining a little too much is preferred to not enough. Remove specimen from acid alcohol and transfer to plain 70% ETOH for 2 hr to remove acid from tissues.

3. *Dehydration.* Transfer specimen to 80% ETOH for 2 hr and then to 95% ETOH for 2 hr. Specimen may be stored overnight or over a weekend in any of these.

4. *Counterstaining.* Make a stock solution of 1% Fast green in 95% ETOH. Add 1 or 2 drops of this stock solution to a BPI or Syracuse watchglass of 95% ETOH. Dip specimen into diluted stain for 1–5 sec while watching under scope. Remove immediately to 95% ETOH and examine. The cuticle of the insect should have a barely perceptible green blush. Repeat if necessary. Fix color by transferring specimen into absolute ETOH. Let set 1–2 hr before clearing. The green will discolor and begin fading if left longer than 2 hr.

5. *Clearing.* Transfer specimen directly into methyl salicylate for approximately 5 min. As soon as the specimen has cleared, remove for mounting.

6. *Mounting.* Mount specimen directly from methyl salicylate into permount, damar balsam, or neutral Canada balsam. Do not use euparal, diatex, or harleco synthetic resin.

The staining procedure was used on dipterous insects, such as mosquito larvae, *Culex pipiens quinquefasciatus* (Say) (Fig. 1), and the larvae of the mushroom fly, *Lycoriella mali* (Fitch) (Fig. 4). Coleopteran larvae, such as the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Fig. 3), and the western corn rootworm, *Diabrotica virgifera virgifera* Le Conte (Fig. 2), were also processed using this staining technique. The mermithid nematode, *Romanomermis culicivorax* (Fig. 1), and the steinernematid nematodes, *Steinernema carpocapsae* (Figs. 2, 3) and *S. feltiae* (Fig. 4), were found to stain well using this technique.

We thank Mrs. Naeema Latif, Nematology Laboratory, Plant Sciences Institute, and Mrs. Patricia Pilitt, Livestock and Poultry Science Institute, for their patience and technical ability, and Mr. Jim Plaskowitz, Botany and Mycology Laboratory, Plant Sciences Institute, for helping to prepare the colored plate.

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